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Prevalence of Chlamydophila psittaci in wild birds—potential risk for domestic poultry, pet birds, and public health?

Zweifel, D ; Hoop, R ; Sachse, K ; Pospischil, A ; Borel, Nicole

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ORIGINAL PAPER

Prevalence of *Chlamydophila psittaci* in wild birds—potential risk for domestic poultry, pet birds, and public health?

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Abstract To determine the prevalence of *Chlamydophila psittaci* in wild birds, cloacal swabs from 527 songbirds, 442 waterfowl, 84 feral pigeons, and 38 cormorants were examined by *Chlamydiaceae*-specific real-time polymerase chain reaction (PCR) and ArrayTube microarray assays for chlamydial species determination and genotyping of *C. psittaci*. Inconclusive cases were further characterized by conventional PCR methods targeting the chlamydial outer membrane protein A, 16S, 23S, and intergenic spacer genes followed by sequencing of the PCR product. Swabs of 19 water birds (tufted ducks and pochards), 12 pigeons, and one songbird were tested positive by the *Chlamydiaceae*-specific real-time PCR. While *C. psittaci* genotypes B (n=5) and E (n=1) were identified in feral pigeons (n=9), the genotype could not be identified in the remaining three cases. Sequence data of *Chlamydiaceae*-positive cases (n=23; 19 waterfowl, three pigeons, one songbird) indicated the presence of nonclassified chlamydial agents (n=20) and *C. psittaci* (n=3) by 16S rRNA PCR and sequencing. In conclusion, *C. psittaci* was not detected in waterfowl and songbirds, but *C. psittaci* proved prevalent in urban feral pigeons, where it poses a significant risk for humans.

Keywords Genotyping · Zoonosis · ArrayTube microarray · Real-time PCR

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Introduction

Chlamydophila psittaci is an obligate intracellular gram-negative bacterium. It shows a unique biphasic reproduction cycle comprising the formation of elementary and reticulate bodies. While the elementary bodies represent the infectious, extracellular, metabolically nonactive forms, the reticulate bodies are the intracellular metabolically active forms. *C. psittaci* was shown to occur in 467 bird species of 30 orders worldwide (Kaleta and Taday 2003). The strains of *C. psittaci* can be subdivided into outer membrane protein A (ompA) gene genotypes. Currently, nine genotypes are generally accepted (A to F, E/B, WC, and M56), and additional six provisional genotypes have been proposed (Sachse et al. 2008a). They differ in their zoonotic potential, virulence traits, and their host preference (Andersen and Tappe 1989; Vanrompay et al. 1993; Andersen 1997; Geens et al. 2005). The disease caused by *C. psittaci* is designated psittacosis in psittacine birds and ornithosis in other bird species. The clinical symptoms include respiratory distress, mucopurulent nasal discharge, diarrhea, polyuria, dullness, keratoconjunctivitis, sinusitis, and central nervous system disturbance depending on the chlamydial genotype involved and the affected bird species (Vanrompay et al. 1995).

In Switzerland, ornithosis/psittacosis is a notifiable disease in birds. Annually, five to ten cases are reported

in captive birds. In contrast to veterinary medicine, psittacosis in humans is not a notifiable disease anymore as the regulation was changed some years ago. From 1941 to 2003, 78 cases in humans were reported (Haag-Wackernagel 2006), due to contact with feral pigeons. Transmission is mainly by inhalation of nasal and fecal secretions. The clinical symptoms in humans are highly variable and range from mild flu-like illness to a severe pneumonia.

At present, little is known about the prevalence of *C. psittaci* in the wild bird population. This lack of knowledge led us to investigate the prevalence of *C. psittaci* in the Swiss wild bird population and to evaluate the potential risk for transmission of *C. psittaci* to domestic poultry, pet birds, and humans.

Materials and methods

Strains of *C. psittaci*

Thirty-one strains (genotype A: $n=27$, genotype B: $n=4$) from the National Reference Centre for Poultry and Rabbit Diseases, University of Zurich, Vetsuisse Faculty, were available for the examinations. They originated mostly from psittacines and pigeons with clinical manifest chlamydiosis from the last 20 years. They had already been genotyped by ompA amplification and restriction fragment length polymorphism (RFLP) analysis by Sudler et al. (2004). The strains were stored in allantoic fluid of inoculated embryonated chicken eggs at −80°C.

Wild birds

Cloacal swabs from 527 songbirds and from 442 waterfowl from the surveillance for avian influenza (September 2005 through January 2006) were investigated. Details of examined bird species are given in Table 1. Samples from songbirds ($n=527$) originated from the northern part of Switzerland, whereas samples from waterfowl ($n=442$) originated from the central part of Switzerland. All samples were stored in buffered peptone water at −80°C.

Feral pigeons

Clinically healthy feral pigeons ($n=60$) from the city of Lucerne, Switzerland, were caught for routine health examination. From these birds, cloacal swabs and formalin-fixed and paraffin-embedded organs (lung, liver, heart, kidney, and spleen) were examined.

In addition, 24 cloacal swabs from clinically healthy feral pigeons from the city of Zurich were examined.

Cormorants

Hunted cormorants ($n=38$) from 2007 to 2008 from a rural region near Zurich, Switzerland, were available. Cloacal swabs and formalin-fixed and paraffin-embedded organs (lung, liver, heart, kidney, and spleen) from each cormorant were examined.

DNA extraction

DNA from the cloacal swabs was extracted using the MagNA Pure® LC System (Roche Diagnostics, Mannheim, Germany), an automated extraction method, according to the manufacturer`s instructions. Two hundred microliters of each sample were used as starting material. From formalin-fixed and paraffin-embedded material, sections of 30–60 μm were cut from each paraffin block and placed into a sterile microcentrifuge tube (one tube per bird). Paraffin was removed by extraction with 1 ml of xylene. After centrifugation at 13,000×*g* for 5 min, residual xylene was removed by twofold extraction with 1 ml of ethanol. Samples were centrifuged (13,000×*g*, 5 min), and ethanol was carefully removed. DNA for polymerase chain reaction (PCR) analysis was extracted from the tissue pellet using a commercial DNA extraction kit (DNeasy Tissue kit, Qiagen, Hilden, Germany).

Real-time PCR assay for *Chlamydiaceae*

All samples were examined on an ABI 7500 instrument (Applied Biosystems, Foster City, CA, USA) using the 23S-based *Chlamydiaceae* family-specific real-time PCR as described previously (Ehricht et al. 2006). The methodology includes primers Ch23S-F (5′-CTGAAACCAGTA GCTTATAAG CGGT-3′), Ch23S-R (5′-ACCTCGCC GTTTAACTTAACTCC-3′), and probe Ch23S-p (FAM-CTCATCA TGCAAAAGGCACGCCG-TAMRA) and yields a 111-bp product specific for members of the family *Chlamydiaceae*. In each reaction, 2.5 μl of extracted DNA was added to a mix of reagents containing 12.5 μl of 2× TaqMan® Fast Universal PCR Master Mix (Applied Biosystems, Darmstadt, Germany), with final concentration of 5 pmol/μl of each primer and the probe (Microsynth, Balgach, Switzerland) to yield a final volume of 25 μl. The cycling profile included initial denaturation (95°C, 10 min) followed by 45 cycles of denaturation at 94°C for 15 s and 60°C for 60 s. A cycle threshold (Ct value) of <38.00 was considered as positive, and all samples were tested at least in duplicate. The results were interpreted as questionably positive if one Ct value was less than 38 and the other showed no Ct value. If one Ct value was above 38 and the other showed no Ct value, the result was interpreted as questionably negative.

Table 1 Details of examined bird species

	Order	Family	Genus	Species
Pigeons (*n*=84)	Columbiformes	Columbidae	*Columba*	Pigeon (*n*=84)
Songbirds (*n*=527)	Passeriformes	Fringillidae	*Fringilla*	Chaffinch (*n*=211)
				Brambling (*n*=107)
			Cuardelis	Siskin (*n*=18)
				Goldfinch (*n*=3)
				Greenfinch (*n*=1)
				Linnet (*n*=1)
			Pyrrhula	Bullfinch (*n*=5)
			Coccthraustes	Hawfinch (*n*=3)
			Serinus	Serin (*n*=1)
		Paridae	*Parus*	Great tit (*n*=47)
			Periparus	Coal tit (*n*=2)
			Cyanistes	Blue tit (*n*=2)
		Turdidae	*Turdus*	Blackbird (*n*=35)
				Song trush (*n*=35)
				Fieldfare (*n*=7)
				Trush (*n*=1)
		Muscipidae	*Erithacus*	Robin (*n*=12)
			Phoenicurus	Black redstart (*n*=6)
		Sturnidae	*Sturnus*	Starling (*n*=11)
		Regulidae	*Regulus*	Goldcrest (*n*=2)
		Sylviidae	*Sylvia*	Blackcap (*n*=2)
		Prunellidae	*Prunella*	Dunnock (*n*=2)
		Corvidae	*Garrulus*	Jay (*n*=1)
		Emberizidae	*Emberiza*	Red bunting (*n*=1)
		Motacillidae	*Motacilla*	Wagtail (*n*=1)
				Unknown species (*n*=10)
Waterfowl (*n*=442)	Anseriformes	Anatidae	*Ayathya*	Tufted ducks (*n*=109)
				Pochard (*n*=103)
				White eye pochard (*n*=3)
		Anas		Mallard (*n*=15)
				Wigeon (*n*=2)
	Charadriiformes	Laridae	*Larus*	Black-headed gull (*n*=67)
				Yellow-headed gull (*n*=21)
				Common gull (*n*=2)
	Pelecaniformes	Phalacrocoracidae	*Phalacrocorax*	Cormorant (*n*=101)
	Gruiformes	Rallidae	*Fulica*	Coot (*n*=48)
			Gallinula	Moorhen (*n*=5)
			Rallus	Water rail (*n*=3)
	Ciconiiformes	Ardeidae	*Ardea*	Grey heron (*n*=1)

ArrayTube microarray identification of chlamydial species

The samples with at least one positive Ct value were examined using the species-specific 23S ArrayTube (AT) microarray assay as described by Borel et al. (2008).

AT microarray assay for ompA genotyping of *C. psittaci*

Samples positive for *C. psittaci* in the AT species identification assay were further examined by the recently developed AT genotyping assay, which was performed as described by Sachse et al. (2008b).

Other conventional PCR methods used and sequencing

Samples positive for *Chlamydiaceae* by real-time PCR, but negative by the AT test for chlamydial species identification, were further examined using other PCR assays targeting chlamydial gene fragments of the 16S, 23S, ompA, and intergenic spacer gene followed by sequencing.

A modified conventional PCR targeting the chlamydial 16S rRNA gene used primer pair 16S-IGF (5′-GAT GAG GCA TGC AAG TCG AAC G-3′) and 16S-IGR (5′-CCA GTG TTG GCG GTC AAT CTC TC-3′; Everett et al. 1999, modified) to amplify a 298-bp product of the 16S rRNA gene specific for the order *Chlamydiales*. One microliter of extracted DNA was added to a PCR mix containing 3.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 μM of each primer (Microsynth), 5 μl of 10× reaction buffer, 2 U of AmpliTaq Gold DNA polymerase (Applied Biosystems), and water to make a final volume of 50 μl. Amplification was performed in 45 cycles with initial denaturation (95°C, 15 min), followed by denaturation at 94°C for 30 s, annealing at 51°C for 30 s, and extension at 72°C for 45 s.

In addition, the 16S rRNA PCR (Everett et al. 1999), the 23S rRNA PCR, the intergenic spacer PCR, and the ompA PCR were used as described by Hotzel et al. (2005).

DNA sequencing

Sequencing of 16S rRNA PCR products was performed in collaboration with the sequencing service of the University of Zurich with an ABI Prism 377 DNA sequencer (Applied Biosystems) or Applied Biosystems 3100 (Synergene Biotech, Zurich, Switzerland). The obtained sequences were compared with the sequences available in GenBank using the BLAST server from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST).

Results

Chlamydial strains

To establish the present diagnostic approach based on a combination of DNA-based methods, we examined the avian chlamydial isolates collected in Switzerland in the last two decades. Besides, it seemed worthwhile to verify our previous genotyping data now using the microarray technology. All chlamydial strains (n=31) were positive for *Chlamydiaceae* by real-time PCR and were identified as *C. psittaci* using the AT species identification assay. Genotype A was determined in 27 out of 31 cases (87%) using the AT genotyping assay, whereas in four out of 31 cases (13%), genotype B was found. The present findings corresponded with the results obtained by RFLP of Sudler et al. (2004).

Feral pigeons

Details of positive results of feral pigeons from Lucerne and Zurich are given in Table 2. A total of 58 out of 60 cloacal swabs from pigeons from Lucerne (96.7%) were tested negative for *Chlamydiaceae* by real-time PCR and AT species identification assay. The remaining two cloacal swabs (3.3%) were positive for *Chlamydiaceae* by real-time PCR and were identified as *C. psittaci* in the AT assay. Further testing using the AT genotyping assay led to

Table 2 Details of positive test results of feral pigeons from Lucerne and Zurich (cloacal swabs)

Case no. (place of origin)	Real-time PCR *Chlamydiaceae* (Ø Ct value)	AT species identification assay	AT genotyping assay	16S rRNA PCR with *C. psittaci*	Homology (%)
1 (Lucerne)	Positive (36.7)	*C. psittaci*	Genotype B	nd	–
2 (Lucerne)	Positive (32.9)	*C. psittaci*	Negative	nd	–
1 (Zurich)	Positive (20.6)	*C. psittaci*	Genotype B	nd	–
2 (Zurich)	Positive (21.8)	*C. psittaci*	Genotype B	nd	–
3 (Zurich)	Positive (22.8)	*C. psittaci*	Genotype B	nd	–
4 (Zurich)	Positive (30.0)	*C. psittaci*	Genotype B	nd	–
5 (Zurich)	Positive (31.5)	*C. psittaci*	Genotype B	nd	–
6 (Zurich)	Positive (31.2)	*C. psittaci*	Genotype E	nd	–
7 (Zurich)	Positive (35.1)	*C. psittaci*	negative	nd	–
8 (Zurich)	Positive (30.1)	Negative	nd	*C. psittaci*	100
9 (Zurich)	Positive (33.7)	Negative	nd	*C. psittaci*	95
10 (Zurich)	Positive (35.0)	Negative	nd	*C. psittaci*	100
11 (Zurich)	Questionably positive (38.3)	Negative	nd	*C. psittaci*	100

nd not done

the identification of genotype B in one case, while the other case gave a negative result. Organs of all 60 pigeons were negative for *Chlamydiaceae* by real-time PCR and AT microarray. In contrast, ten out of 24 (41.7%) pigeons from Zurich proved positive by the 23S-based family-specific real-time PCR and one cloacal swab was questionably positive. *C. psittaci* could be identified by the species-specific AT assay in seven cases, out of which five cases were genotype E, one case was genotype B, and one case could not be identified by AT genotyping assay. As an illustration, the results of testing a pigeon sample from Zurich using real-time PCR, AT species identification, and genotyping for *C. psittaci* are shown in Fig. 1. Real-time PCR-positive or questionably positive, but AT assay-negative (n=4), cases were further examined by PCR targeting the ribosomal 16S and 23S rRNA genes, the ompA gene, and the intergenic spacer gene. Sequencing of 298-bp products of 16S rRNA PCR (Everett et al. 1999, modified) revealed 95–100% homology to *C. psittaci* in all four cases.

Songbirds

Five hundred twenty-five out of 527 (99.6%) cloacal swabs from wild songbirds were negative for *Chlamydiaceae* by real-time PCR, whereas one sample was positive for *Chlamydiaceae* by real-time PCR, but negative in the AT assay, and another swab gave rise to a questionably negative result by real-time PCR. These two samples were further investigated by other PCR methods as described, but positive bands at 298 bp were only detected by 16S rRNA PCR (Everett et al. 1999, modified) in both *Chlamydiaceae* real-time positive, but AT assay negative cases. Both cases were negative with the ompA, 23S, and intergenic spacer conventional PCR methods. Sequencing of the PCR product demonstrated 92% to *Chlamydia suis* in one case, and no meaningful homology to any member of the family *Chlamydiaceae* in the other case.

Waterfowl

Altogether, 423 out of 442 cloacal swabs (96%) were negative for *Chlamydiaceae* by real-time PCR, whereas 19 swabs (4%) showed a positive result (Ct values ranging from 30.1 to 37.0), but the chlamydial species could not be identified by the AT microarray. Thus, these 19 cases were further examined by PCR methods targeting other chlamydial genes (chlamydial 16S rRNA, 23S rRNA, intergenic spacer, and ompA genes) as described. Positive bands at 298 bp were obtained in all cases using 16S rRNA PCR (Everett et al. 1999, modified). Sequencing of the PCR products indicated the presence of a nonclassified chlamydial agent with only 92% (15 cases), 91% (three cases), and 90% (one case) homology to *C. suis* as the closest relative. Bird species with positive 16S rRNA PCR results (n=19) were tufted ducks (n=12) and pochards (n=7).

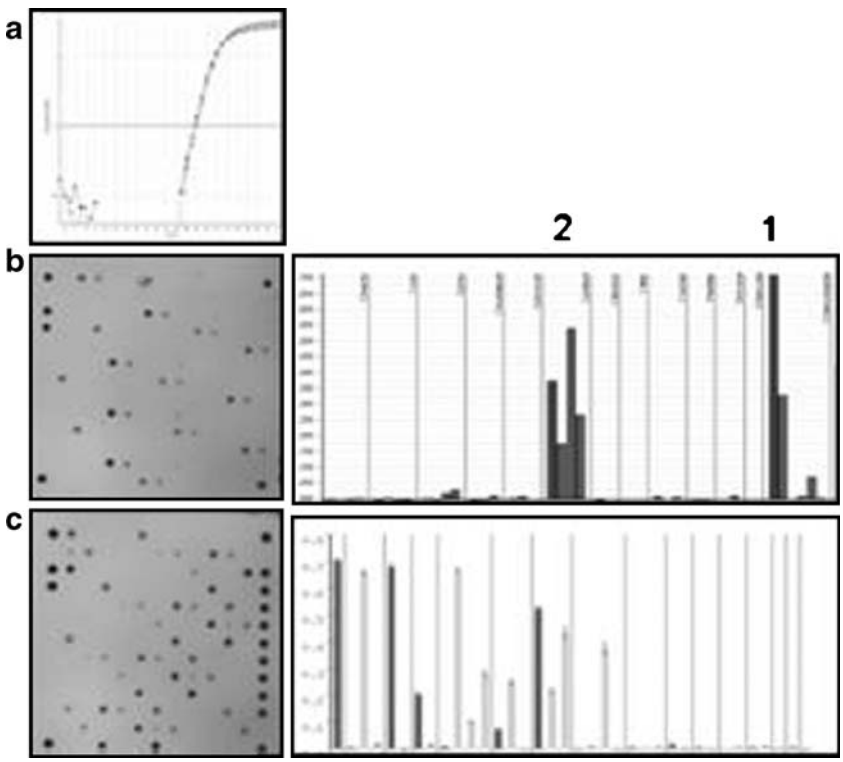

Fig. 1 DNA-based examination of sample T21 (cloacal swab from a feral pigeon from Zurich) using three different tests. **a** Amplification plot of real-time PCR assay (23S-rtPCR) for family *Chlamydiaceae* (mean Ct=30.0). **b** AT species identification assay. *Left* microarray image; *right* barplot showing specific signals for genus *Chlamydophila* (*1*) and species *C. psittaci* (*2*). **c** AT genotyping assay. *Left* microarray image; *right* barplot showing typical hybridization pattern of *C. psittaci* genotype B

Cormorants

All cloacal swabs and organs from the investigated cormorants (n=38) were negative for *Chlamydiaceae* by real-time PCR.

Discussion

The aim of the study was to investigate the prevalence of *C. psittaci* in the Swiss wild bird population and to evaluate the potential risk for transmission of ornithosis to domestic poultry, pet birds, and humans. In the first part of the study, we established the methodological approach for examining wild bird samples on 31 *C. psittaci* strains. We screened all 31 samples by real-time PCR specific for *Chlamydiaceae* and positive cases were further evaluated by AT species identification assay and AT genotyping assay. This approach allowed us to screen a large number of samples by real-time PCR, followed by specific microarray methods to detect *C. psittaci* and identify its genotypes, which represents a procedure suitable for routine diagnostic analyses (Sachse et al. 2005, 2008a, b; Borel et al. 2008). The present genotyping results were fully concordant with the results of Sudler et al. (2004) obtained by RFLP, which shows equal suitability of both methods for diagnosing ornithosis cases. However, RFLP genotyping can distinguish only among six genotypes, whereas AT genotyping was shown to identify at least eight different types (A, B, C, D, E, E/B, WC, and M56) and allow testing directly from clinical samples (Sachse et al. 2008a, b). Genotype A was identified in 87% and genotype B in 13% of Swiss cases of avian chlamydiosis (parrots and pigeons). These results correlated to previous findings, where genotype A was predominantly found in psittacine birds, and genotype B was the second most frequent genotype in Belgium, Switzerland, Germany, UK, and The Netherlands (Vanrompay et al. 1997).

Furthermore, cloacal swabs and organs from feral pigeons from two different cities of Switzerland were examined. The prevalence of *C. psittaci* differed significantly between these two groups: Only two (3.3%) of 60 cloacal swabs from pigeons of Lucerne were positive for *C. psittaci*; genotype B could be identified in one case. In contrast, ten (41.7%) of 24 cloacal swabs were *Chlamydiaceae* positive and one was questionably positive in pigeons from Zurich. From these 11 cases, seven (63.6%) were positive for *C. psittaci*, and genotype B was found in five cases and genotype E in one case. The chlamydial species could not be identified by AT microarray assay in the remaining three cases, although subsequent 16S rRNA PCR and direct sequencing of these three cases revealed 95–100% homology to *C. psittaci*. There can be several explanations why the chlamydial species could not be identified by AT microarray assay and AT genotyping but by 16S rRNA PCR. The amount of chlamydial DNA in the cloacal swabs was probably low as these samples originated from clinically healthy pigeons, so that it was below the detection limit of the AT assay (sensitivity for clinical samples=0.81, Borel et al. 2008), but still detectable by the more sensitive 16S rRNA PCR (sensitivity for clinical samples=0.84, Borel et al. 2008). Samples positive for *C. psittaci* by 16S rRNA PCR, but not positive in the AT genotyping assay, either contain too low DNA amounts or may belong to aberrant genotypes. Although only limited numbers of pigeons were investigated, a difference in prevalence depending on the region was discovered. These findings correlate with other studies, e.g., the review by Magnino et al. (2008), where prevalences for *C. psittaci* in different European cities ranged from 0% in Zagreb to 57.1% in Paris. Heddema et al. (2006) reported hints for a seasonal influence on chlamydial infections. They found more chlamydial infected pigeons during the breeding season (18/171) than during the low-breeding season (8/160). This is in contrast to our study, where pigeons from Lucerne were caught in May 2007 and the pigeons from Zurich in September 2008. Thus, the marked difference in the *C. psittaci* prevalence between these two cities cannot be explained by the breeding season. The predominance of genotype B in pigeons from Zurich correlated with previous studies (Vanrompay et al. 1997). All tested organs from healthy pigeons from Lucerne (n=60) were negative, which indicates asymptomatic intestinal infection with cloacal shedding. Although these birds were clinically healthy, excretion of *C. psittaci* is always possible. Such positive pigeons pose a risk for transmission of *C. psittaci* to humans especially in cities, where close contact to infected animals is possible (as often, large numbers of pigeons are crowded on a small feeding place). Several cases of zoonotic disease in humans transmitted by pigeons have been reported in the past (Haag-Wackernagel 2006). In cities, risk for transmission from pigeons to pet birds is low, as pet birds are often kept in houses, not having contact to feral pigeons or their excretions. In contrast to the pigeons, the examined cormorants (n=38) originated from a rural region in the northern part of Zurich. Both organs and cloacal swabs were negative for *Chlamydiaceae* indicating that cormorants are not frequently affected by *C. psittaci*, although sample numbers were low. Nowadays, scarce evidence from the literature is available on the prevalence of *C. psittaci* in cormorants. A study by Travis et al. (2006) reported antibodies to *C. psittaci* in flightless cormorants from Galapagos (11%).

Surprisingly, *C. psittaci* was not detected in samples from 527 songbirds and 442 waterfowl. This suggests that they do not pose a risk for transmission of ornithosis to other bird species. *Chlamydiaceae*-positive results were

found in only two songbirds and 19 waterfowl by real-time PCR. The AT microarray of these was negative, but the 16S rRNA PCR and sequencing, resulted in a 92% (16 cases), 91% (three cases), and 90% (one case) homology to *C. suis*. Everett et al. (1999) published the new taxonomic classification in 1999 and defined that an identity of >90% justifies classification within the family *Chlamydiaceae*, an identity of >99.2% justifies classification within the same species, whereas an identity of >97% means assignment to the same genus. Thus, new yet unidentified chlamydial organisms are likely to be present in our samples. The present data suggest that the so far, nonclassified chlamydial agent found in songbirds and waterfowl should belong to the family *Chlamydiaceae* with *C. suis* being its closest relative. Culturing and further identification of these so far nonclassified chlamydial organisms is needed in the future.

In conclusion, no significant risk for transmission of *C. psittaci* from wild songbirds and waterfowl to humans and other birds was identified, but a moderate risk associated with feral pigeons has to be taken into account in urban areas.

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References

Andersen AA (1997) Two new serovars of *Chlamydia psittaci* from North American birds. J Vet Diagn Invest 9:159–164

Andersen AA, Tappe JP (1989) Genetic, immunologic, and pathologic characterization of avian chlamydial strains. J Am Vet Med Assoc 195:1512–1516

Borel N, Kempf E, Hotzel H, Schubert E, Torgerson P, Slickers P, Ehricht R, Tasara T, Pospischil A, Sachse K (2008) Direct identification of Chlamydiae from clinical samples using a DNA microarray assay: a validation study. Mol Cell Probes 22:55–64. doi:10.1016/j.mcp.2007.06.003

Ehricht R, Slickers P, Goellner S, Hotzel H, Sachse K (2006) Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies. Mol Cell Probes 20:60–63. doi:10.1016/j.mcp.2005.09.003

Everett KD, Bush RM, Andersen AA (1999) Emended description of the order *Chlamydiales*, proposal of *Parachlmydiaceae* fam. nov. and *Simkaniaceae* fam. nov., each containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species, and standards for the identification of organism. Int J Syst Bacteriol 49:415–440

Geens T, Desplanques A, Van Loock M, Bönner BM, Kaleta EF, Magnino S, Andersen AA, Everett KD, Vanrompay D (2005) Sequencing of the *Chlamydophila psittaci* ompA gene reveals a new genotype, E/B, and the need for a rapid discriminatory genotyping method. J Clin Microbiol 43:2456–2461. doi:10.1128/JCM.43.5.2456-2461.2005

Haag-Wackernagel D (2006) Human diseases caused by feral pigeons. In: Feare CJ, Cowan DP (eds) Advances in vertebrate pest management, vol 4. Filander, Fürth, pp 31–58

Heddema ER, Ter Sluis S, Buys JA, Vandenbroucke-Grauls CM, van Wijnen JH, Visser CE (2006) Prevalence of *Chlamydophila psittaci* in fecal droppings from feral pigeons in Amsterdam, The Netherlands. Appl Environ Microbiol 72:4423–4425. doi:10.1128/AEM.02662-05

Hotzel H, Blahak S, Diller R, Sachse K (2005) Evidence of infection in tortoises by Chlamydia-like organisms that are genetically distinct from known *Chlamydiaceae* species. Vet Res Commun 29:71–80. doi:10.1007/s11259-005-0838-y

Kaleta EF, Taday EM (2003) Avian host range of *Chlamydophila* spp. based on isolation, antigen detection and serology. Avian Pathol 32:435–461. doi:10.1080/0307945031000159361 3

Magnino S, Haag-Wackernagel D, Geigenfeind I, Helmecke S, Dovč A, Prukner-Radovčić E, Residbegović E, Ilieski V, Laroucau K, Donati M, Martinov S, Kaleta EF (2008) Chlamydial infections in feral pigeons in Europe: review of data and focus on public health implications. Vet Microbiol 135:54–67

Sachse K, Hotzel H, Slickers P, Ellinger T, Ehricht R (2005) DNA microarray-based detection and identification of *Chlamydia* and *Chlamydophila* spp. Mol Cell Probes 19:41–50. doi:10.1016/j.mcp.2004.09.005

Sachse K, Laroucau K, Hotzel H, Schubert E, Ehricht R, Slickers P (2008a) Genotyping of *Chlamydophila psittaci* using a new DNA microarray assay based on sequence analysis of *omp*A genes. BMC Microbiol 8:63. doi:10.1186/1471-2180-8-63

Sachse K, Laroucau K, Vorimore F, Magnino S, Feige J, Müller W, Kube S, Hotzel H, Schubert E, Slickers P, Ehricht R (2008b) DNA microarray-based genotyping of *Chlamydophila psittaci* strains from culture and clinical samples. Vet Microbiol 135:22–30

Sudler C, Hoelzle L, Schiller S, Hoop RK (2004) Molecular characterisation of chlamydial isolates from birds. Vet Microbiol 98:235–241. doi:10.1016/j.vetmic.2003.07.008

Travis EK, Vargas FH, Merkel J, Gottdenker N, Miller RE, Parker PG (2006) Hematology, plasma chemistry, and serology of the flightless cormorant (*Phalacrocorax harrisi*) in the Galapagos Islands, Ecuador. J Wildl Dis 42:133–141

Vanrompay D, Andersen AA, Ducatelle R, Haesebrouck F (1993) Serotyping of European isolates of *Chlamydia psittaci* from poultry and other birds. J Clin Microbiol 31:134–137

Vanrompay D, Ducatelle R, Haesebrouck F (1995) *Chlamydia psittaci* infections: a review with emphasis on avian chlamydiosis. Vet Microbiol 45:93–119. doi:10.1016/0378-1135(95)00033-7

Vanrompay D, Butaye P, Sayada C, Ducatelle R, Haesebrouck F (1997) Characterization of avian *Chlamydia psittaci* strains using omp1 restriction mapping and serovar-specific monoclonal antibodies. Res Microbiol 148:327–333. doi:10.1016/S0923-2508(97)81588-4